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Letter to the Editor

+2.71 LOD Score at Zero Recombination Is Not Sufficient for Establishing Linkage Between X-Linked Mental Retardation and X-Chromosome Markers

To the Editor:

Nonspecific X-linked mental retardation (MRX) is the denomination attributed to the familial type of mental retardation compatible with X-linked inheritance but lacking specific phenotypic manifestations [reviewed in Schwartz, 1993]. It is thus to be expected that families falling under such broad definition are genetically heterogeneous in the sense that they may be due to different types of mutations occurring, most probably, at distinct X-chromosome loci.

To facilitate a genetic classification of these conditions, the Nomenclature Committee of the Eleventh Human Gene Mapping Workshop proposed to assign a unique MRX-serial number to each family where evidence of linkage with one or more X-chromosome markers had been established with a LOD score of at least +2 at zero recombination [Mulley et al., 1992]. This letter is meant to emphasize the inadequacy of this criterion for a large pedigree where the segregation of the disease has been evaluated against the haplotype constitution of the entire X-chromosome carrying the mutation in question.

The three-generation Sardinian pedigree (Renp-1) shown in Figure 1 is an updated version of a previously described family segregating for a syndromal type of X-linked mental retardation known as Renpenning syndrome [Archidiacono et al., 1987]. Search for linkage was carried out by following the segregation of the disease phenotype vs. that of 16 highly informative polymorphic loci spanning the X-chromosome from Xp22.3–Xq28. The segregation data and pairwise LOD score values obtained from this analysis are given in Figure 2 and Table I, respectively. As specified in the legend to Figure 2, the genetic phase of the female progenitor I-1, at each of the reported loci, has been established by somatic cell hybridization. From the LOD score table, it is clear that the existence of an absolute linkage with the disease can definitely be ruled out for the 12 pairwise comparisons yielding a minus infinite LOD score value at zero recombination. On the other hand, the existence of close linkage is suggested between the disease locus and the remaining two pairs of markers (PFC/SYNARAF1 and DXS456/DXS424), both yielding a maximum LOD score greater than the recommended +2 threshold, even though the two pairs of markers in question are located in chromosomal regions (Xp11.3–p11.23 and Xq21–q26, respectively) that are at a nonmeasurable genetic distance from one another [Willems et al., 1993; Gendrot et al., 1994]. Another interesting finding in this family is that the female progenitor I-1 inherited the Renp-1 mutation from her father, who is reported as being entirely normal.

The following considerations are an attempt to explain the above-mentioned unexpected findings:

1) Evidence of close linkage must be erroneous for one of the two sets of marker loci, probably due to the occurrence of a double recombination event between the disease locus and one pair of the alleged closely-linked markers.

2) If this is the case, it is more likely that the false linkage is the one between the disease and the pair of

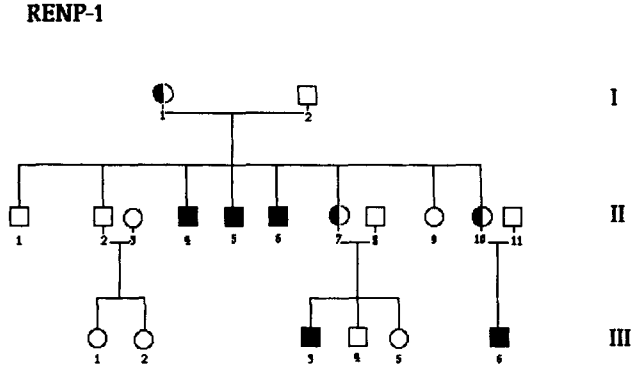

Fig. 1. Pedigree of Renp-1 family. Detailed clinical data are reported in Archidiacono et al. [1987].

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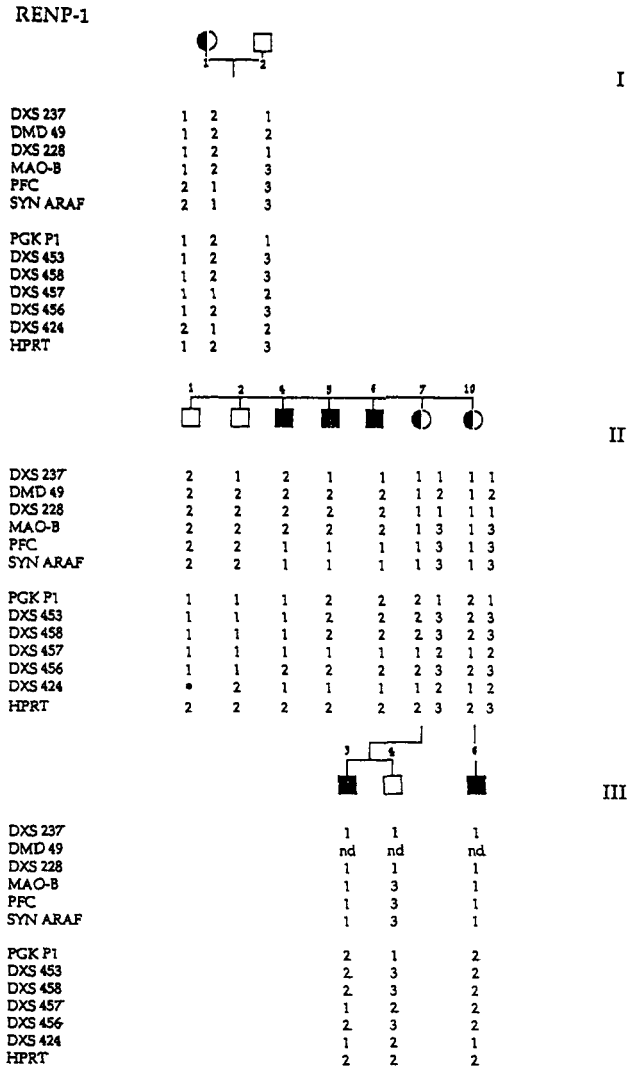

Fig. 2. Segregation data. Only the relevant part of the pedigree is shown. Following PCR amplification, the different-length alleles [Weber and May, 1989] were resolved on nondenaturing polyacrylamide (8% w/v) gel electrophoresis, after ethidium bromide staining. Genetic phase for individual I-1 was unequivocally established by determining individual microsatellite markers on somatic cell hybrid clones which had retained only one X chromosome. This was achieved by PEG-induced fusion of peripheral blood lymphocytes from individual I-1 with HPRT-deficient rodent cells followed by selection of HAT-resistant clones, DNA preparation, and PCR amplification. nd, not determined; *, repeatedly failed to amplify.

long-arm markers, because the likelihood of crossovers (and, even more so, of double crossovers) in the pericentromeric region of the short arm is known to be very low. This conclusion is consistent with reported preliminary data [Stevenson et al., 1994] that located the locus of Renpenning syndrome in the pericentromeric region.

3) Evidence of close linkage could actually be false with respect to both sets of markers, and therefore a true linkage with the disease remains to be found.

4) The disease might be the result of a fresh mutation which occurred in the spermatogenesis of the progenitor's (I-1) father, or the latter could be the carrier of

TABLE I. Two-Point Linkage Analysis Between Renp-1 Locus and Markers on X-Chromosome*

		LOD score at theta								
Locus	Location	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Z max	θ max#
DXS237	Xp22.3	−∞	−2.22	−0.91	−0.42	−0.07	0.02	0.01	0.02	0.3
DXS207	Xp22.2	−∞	−2.8	−1.44	−0.89	−0.39	−0.15	−0.04	−0.04	0.4
DXS451	Xp22.3–p21.2	−∞	−2.8	−1.44	−0.89	−0.39	−0.15	−0.04	−0.04	0.4
DMD49	Xp21.3–21.1	−∞	−4.21	−2.16	−1.33	−0.58	−0.23	−0.05	−0.05	0.4
DXS228	Xp11.4–p11.3	−∞	−4.21	−2.16	−1.33	−0.58	−0.23	−0.05	−0.05	0.4
MAOB	Xp11.4–p11.3	−∞	−3.32	−1.33	−0.57	0.03	0.21	0.18	0.21	0.3
PFC	Xp11.3–p11.23	2.71	2.67	2.49	2.25	1.74	1.16	0.52	2.71	0
SYN/ARAF1	Xp11.3–p11.23	2.71	2.67	2.49	2.25	1.74	1.16	0.5	2.71	0
PGKP1	Xq11.2–q12	−∞	0.67	1.21	1.3	1.14	0.8	0.37	1.3	0.1
DXS453	Xq12	−∞	0.67	1.21	1.3	1.14	0.8	0.37	1.3	0.1
DXS458	Xq21.1–q22	−∞	0.67	1.21	1.3	1.14	0.8	0.37	1.3	0.1
DXS457	Xq21.2–q22	−∞	−1.11	−0.44	−0.19	0.01	0.07	0.06	0.07	0.3
DXS456	Xq21.33–q22	2.71	2.67	2.49	2.25	1.74	1.16	0.5	2.71	0
DXS424	Xq24–q26	2.41	2.37	2.21	2	1.54	1.02	0.45	2.41	0
HPRT	Xq26.1	−∞	−3.32	−1.35	−0.61	−0.06	0.09	0.08	0.09	0.4
G6PD	Xq28	−∞	−3.32	−1.35	−0.61	−0.06	0.09	0.08	0.09	0.4

* Polymorphic loci spanning the X-chromosome from Xp22.3–Xq28. Analysis was carried out with the MLINK program of the FASTLINK package (version 2.2) [Lathrop et al., 1985; Cottingham et al., 1993; Schaffer et al., 1994].

a premutation, as happens in other inherited conditions such as Martin-Bell syndrome [Oberlé et al., 1991] and myotonic dystrophy [Shelbourne et al., 1992].

Whatever the correct interpretation of our findings, the clear message stemming from this report is that a LOD score of +2 at zero recombination is not a definitive, but only a tentative, localization. Investigators must examine all markers along the chromosome arm and interpret LOD score simultaneously.

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